16 Microbiological and Biochemical Aspects of the Rhizosphere

A. D. ROVIRA and
BARBARA M. McDOUGALL
CSIRO
Division of Soils
Adelaide, South Australia

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16-1. INTRODUCTION

The microbial population of soil is altered both quantitatively and qualitatively by the presence of plant roots. The zone of soil in which this influence is extended is called the "rhizosphere." While recent reviews (*1,2*) on the rhizosphere have emphasized its microbiological aspects, this chapter will concern itself chiefly with the activities of roots and microorganisms in situ and their mutual interactions, specifically their noninvasive associations; hence, it will not attempt to cover the important symbiotic associations between roots and fungi in endotrophic and ectotrophic mycorrhizas or between legumes and rhizobia.

The rhizosphere is not a uniform or well-defined part of the soil; instead, it represents a poorly defined zone with a microbiological gradient, in which the maximal effect of roots on microorganisms is in the soil nearest the root. Any consideration of the rhizosphere includes the microorganisms that colonize the root surface and are not a true part of the soil population. The terms "outer rhizosphere," "inner rhizosphere," "root surface," and "rhizoplane" have been used to define the zones of influence, but until recently most studies of the interactions between plant roots and soil microorganisms have not attempted to distinguish between the rhizosphere and the rhizoplane. Throughout this review the term "rhizosphere" will be used in connection with those microorganisms both on the root and in that soil which is influenced by the root; the term "rhizoplane" will be used specifically in connection with organisms that develop on the roots themselves and are not in the soil.

16-2. ASSESSMENT OF QUANTITATIVE CHANGES IN THE MICROFLORA OF THE RHIZOSPHERE

In most studies of the effects of plant roots upon soil microorganisms some samples of roots with firmly adhering soil are shaken in sterile diluent, a dilution series is prepared, and aliquots are transferred to petri dishes into which is poured a nonselective agar medium. The control samples comprise soil from beyond the rhizosphere, such as fallow soil or soil between plants, and plate counts are prepared from suspensions of these samples. The R/S ratio, which is the ratio of the numbers of organisms per gram of rhizosphere soil included with the root tissue to the numbers per gram of control soil, has been widely used to express the extent to which the plant roots affect the numbers of microorganisms (*3*). When one considers that the density of microorganisms is greatest at the root surface and declines with distance from the root, it is not surprising to find that the R/S ratio of replicate plants can be made to change considerably by varying the amount of soil adhering to the roots suspended in diluent (*4–6*). For this reason caution is required when the R/S ratios are used for comparing

the rhizosphere effects of different plants or the results from different laboratories.

A more satisfactory method of studying quantitative changes in the rhizosphere is that of Ishizawa et al. (*7*) and Louw and Webley (*8*), who defined the rhizosphere as that soil which is removed when roots and firmly adhering soil are *gently* shaken in sterile water, and the rhizoplane as that soil which is obtained when the washed roots are transferred to a fresh dilution flask and shaken vigorously. Although by this method some of the rhizosphere microorganisms will be counted in the rhizoplane sample and vice versa, it undoubtedly gives the best assessment of the degree of microbial change effected by roots. In comparisons of the effects on microflora of different plant species the results should be expressed on a root-weight basis for the rhizoplane populations and on a soil-weight and root-weight basis for the rhizosphere populations.

The serial washing technique, in which the roots are washed vigorously in sterile water several times before fragmentation or maceration and dispersion in agar, has been used for fungi (*9,10*) and bacteria (*11*), including *Azotobacter* (*12*). Although this method reveals the organisms that are firmly held or embedded in the root and is the best method for studying root-colonizing fungi because it eliminates most of the fungal spores (*9*), the gentle root-washing technique is probably best for studying bacteria.

These culturing techniques for quantitatively assessing the rhizoplane and rhizosphere populations provide the isolates necessary for studying the microflora that predominates in the rhizosphere.

16-3. DIRECT OBSERVATION OF THE RHIZOPLANE AND RHIZOSPHERE MICROFLORA

The direct examination of roots under the microscope has not been widely used, because it renders little quantitative information, but it is valuable for studying the actual distribution of microorganisms on the root surface. By staining washed roots with a mixture of aniline blue, phenol, and acetic acid (*13*) it is possible to observe the bacteria, fungi, and actinomycetes. This method has shown that roots of oats and tomato are colonized by bacteria within six hours of emergence from the seed (*14*); the root tip was almost always found devoid of bacteria, which first appeared as isolated cells or small clusters in the zone of elongation, but the older root portions, where the root surface was densely colonized often had bacteria many cells deep. Different patterns of development in the rhizoplane microflora have been observed by this method: on *Phalaris tuberosa* L. roots bacteria were clustered in colonies containing 8 to 100 or more cells, and on tomato roots bacteria and often fungi aligned themselves along the axis of the root (*2*).

Fig. 16-1. Development of bacteria in mucilaginous material at base of root hair of 4-day-old tomato (× 750).

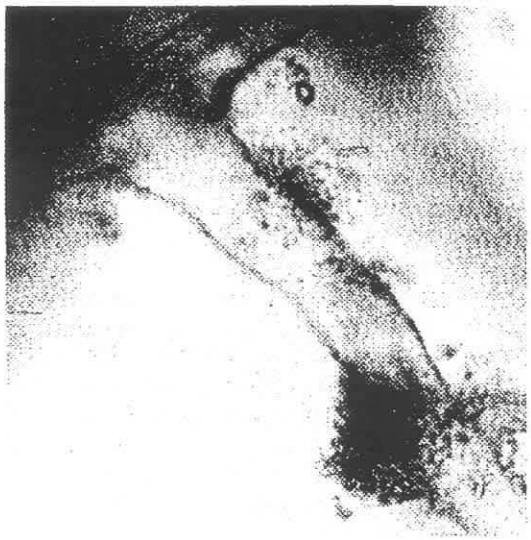

Fig. 16-2. Bacterial colonies on a root hair of 4-day-old tomato (× 750).

Figures 16-1 to 16-6 show the bacterial colonization of young wheat, tomato, and subterranean clover roots grown in sand with plant-nutrient solution.

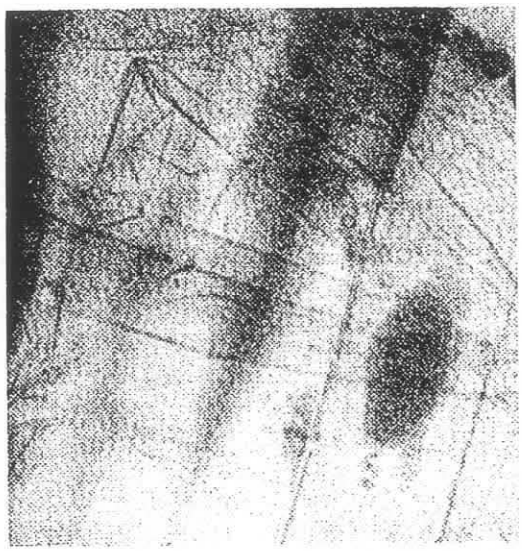

Fig. 16-3. Development of bacterial colonies on a root of 4-day-old subterranean clover (× 750).

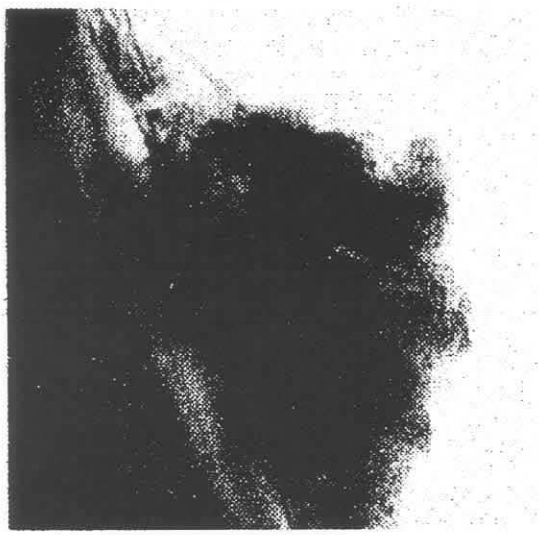

Fig. 16-4. Bacterial colony projecting from the root surface of 6-day-old wheat (× 750).

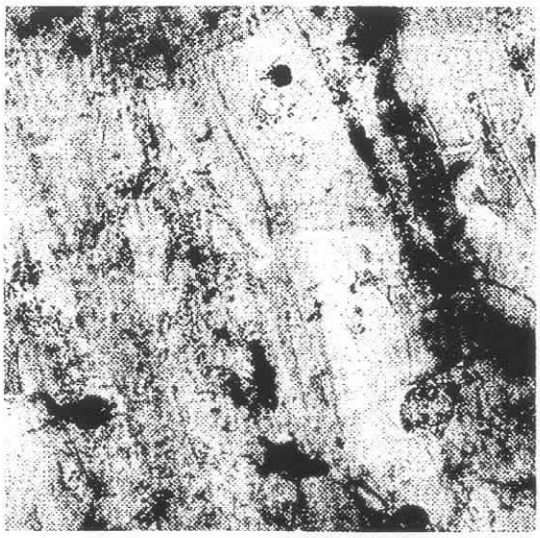

Fig. 16-5. Bacteria on a root of 14-day-old wheat (×600).

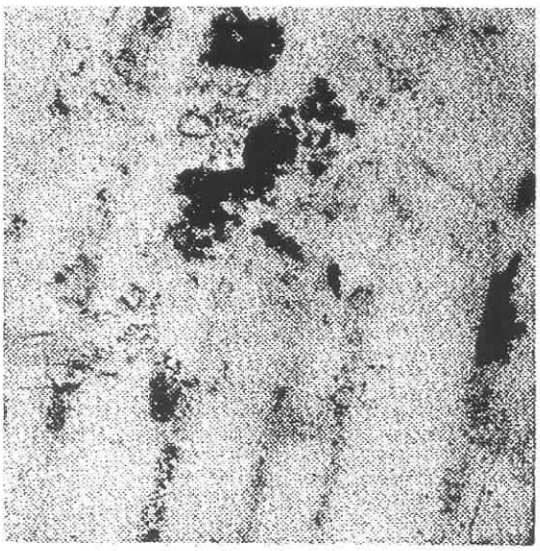

Fig. 16-6. *Azotobacter* and other bacteria on a root of 14-day-old wheat grown from *Azotobacter*-inoculated seed (×600).

Direct microscopy of roots of corn, cabbage, and beans has shown that fungal hyphae are seldom observed in the root tip region but become more abundant towards the crown, although even there they cover only a portion of the root surface (*10*).

Light microscopy has revealed that there are often many layers of bacteria overlying the root. This was convincingly confirmed when sections of barley roots and medic roots with intact rhizoplane and inner rhizosphere were examined under the electron microscope (*15,16*).

The technique developed by Jones and Griffiths (*17*), of impregnating soil with resin and grinding it down to sections 20 to 30 μ thick, for studying the distribution of microorganisms throughout soil crumbs, could be applied to rhizosphere studies. Although it is tedious and only a few specimens may be examined, it has enabled the authors to demonstrate definite patterns of distribution of bacterial colonies in aggregates, and it might be useful in delimiting the extent of the rhizosphere.

16-4. NATURE OF THE RHIZOSPHERE EFFECT

A. Quantitative Aspects

When plant roots grow through soil there is a marked increase in the numbers of bacteria, fungi, and actinomycetes in the soil near the roots.

1. Bacteria

It is not uncommon to find reports in the literature of R/S ratios as high as 100 for bacteria. More commonly they range from 5 to 20, but in any application it should be remembered that the R/S ratio is not an absolute figure, being dependent upon the soil type, root system, and the manipulation of the sample. Comparisons made by Louw and Webley (*8*) of direct microscopical counting and plate counting with a nonselective medium showed that the ratio of direct count to plate count was lowest in the rhizoplane soil and highest in the control. Table 16-1, prepared from the data of Louw and Webley (*8*), shows that this ratio became less as the plants aged.

It is not likely that these results are due to a lower proportion of viable cells in the control soil but, rather, that the roots selectively stimulate those bacteria capable of utilizing the nutrients of root exudates and that these organisms are better adapted to artificial media. On the other hand, the majority of soil organisms will not develop on artificial agar media, although they may be viable and capable of growth when given appropriate nutrients. This has been demonstrated by Casida (*18*), who found that by inoculating aliquots from 10^{-8} and 10^{-9} dilutions of samples of different

TABLE 16-1
Ratios of Direct Count to Plate Count of Control Soil, Oat Rhizosphere, and Oat Rhizoplane (*8*)

Plant age, wk	Ratio of direct count to plate count		
	Control	Rhizosphere	Rhizoplane
16	11.3	5.1	5.2
17	9.2	3.1	2.6
20	7.9	1.4	1.2

soils into heart infusion broth he could isolate bacteria incapable of growth on normal soil-extract agar media. These organisms were catalase-negative, microaerophilic, coccoid bacteria, possibly related to the *Actinomycetaceae* or *Mycobacteriaceae*, and they certainly outnumbered the bacteria that grow on agar plates.

2. Fungi

The filamentous nature of fungi and the prolific sporulation of certain species make this group more difficult to assess quantitatively than the bacteria. Katznelson (*19*) quotes R/S ratios of 10 and 19 for fungi from wheat and mangels, respectively, but these figures were obtained by the standard dilution procedure, which estimates the numbers of fungal spores rather than the numbers or quantity of active mycelia.

Although it is difficult to give quantitative assessments of the effects of roots on fungi existing in soil as mycelia, there is undoubtedly an effect upon these fungi, which is indicated by the qualitative differences between soil and rhizoplane isolates. Harley and Waid (*20*) showed that the frequency of isolation of fungi from segments of washed roots increased with the age of the root; this finding was later confirmed by direct microscopic examination of roots (*10*).

Although direct microscopy shows that fungal filaments are not as abundant as bacteria on roots, what should be considered in any quantitative assessment of the relative importance of these groups in the rhizosphere is the quantity of cell material. Fungi, having larger size and filaments, require few units to be equivalent to many thousands of bacterial cells. However, no estimates have been made of the relative amounts of fungal and bacterial cell material in the rhizosphere, for obvious technical reasons.

3. Actinomycetes

The problems of estimating the numbers of actinomycetes in the rhizosphere are similar to those involved in counting sporing fungi, but it is

generally accepted that roots stimulate actinomycetes to a lesser extent than bacteria. The actinomycetes merit special attention, because many of them produce antibiotics and are antagonistic to bacteria. Rouatt et al. (*21*) found that rhizospheres of wheat, oats, lucerne, soybeans, mangels, and potatoes supported proportionally more actinomycetes antagonistic to bacteria than did control soil. Strzelczyk (*22*) reported similar results, a larger proportion of actinomycetes isolated from wheat, radish, and onion rhizospheres being antagonistic to *Azotobacter* than of actinomycetes isolated from soil beyond the rhizosphere.

4. Algae and Protozoa

There are conflicting reports on the influence of plant roots on algae and protozoa, some authors claiming stimulation and others none. In the case of protozoa there would be an indirect effect in that extra foodstuff would be provided by the more numerous bacteria of the rhizosphere.

5. Nematodes

Free-living nematodes are more abundant in the rhizosphere than in soil beyond it (*23*), the R/S ratios ranging from 13 to 71 in the rhizospheres of various plant species (*24*).

B. Qualitative Aspects

In the preceding section evidence was produced that the density of the microbial population of the rhizosphere is considerably greater than that of the soil beyond the influence of the roots. However, this greater density is not due simply to a general stimulation of the soil population by roots, for certain species of the population are stimulated to a greater degree than others.

1. Bacteria

There is a selective stimulation of Gram-negative rods by plant roots, so that these form greater proportions of the rhizosphere than the soil populations. This is not simply a matter of growth rate or of ability to withstand competition, as was shown when pure cultures of *Bacillus polymyxa*, *Clostridium pasteurianum*, *Azotobacter chroococcum*, and *Pseudomonas fluorescens* were inoculated singly into sand in which plants were growing aseptically (Table 16-2).

The results of Krasil'nikov (*26*) demonstrate that bacteria which colonize the rhizosphere of one plant species do not necessarily colonize that of other species and also that not all strains of *Pseudomonas fluorescens* have equal colonizing abilities. The results in Table 16-3 were given by Krasil'nikov to show the effects of plants on the qualitative composition of bacteria

TABLE 16-2

Proliferation of Pure Cultures of Bacteria in the Rhizospheres of Various Plants (*25*)

	Number of bacteria per root system after two weeks ($\times 10^3$)			
	Lucerne	Maize	Tomato	Wheat
Azotobacter chroococcum	1	1	40	1
Bacillus polymyxa	800	8,000	360	16,000
Clostridium pasteurianum	400	4,000	320	3,200
Pseudomonas fluorescens	5,000	800,000	4,000	80,000

in the rhizosphere when five bacterial species (whether singly or mixed is not made clear) were inoculated into sterile Knop's nutrient solution in which the plants were growing.

Krasil'nikov also reported that similar experiments with sporing bacteria showed that the Gram-positive sporing rods *Bacillus mesentericus*, *B. subtilis*, and *B. megatherium* did not grow in the solution. However, the roots were not lethal to them, for the inoculum survived.

Chan and Katznelson (*27*) have shown, however, that competition or antagonism can affect the development of organisms in root extract. They found that *Arthrobacter globiformis* and *Pseudomonas* sp. had almost

TABLE 16-3

Growth of Bacteria in Knop's Solution in the Presence of Plant Roots (*26*)

	Number of bacteria per milliliter after 20 days ($\times 10^3$)					
	Wheat	Cotton	Sugar beet	Clover	Lucerne	Peas
Rhizobium trifollii	10	10,000		100,000	1,000	10,000
Rhizobium meliloti	100	100,000	1,000	10,000	100,000	1,000
Azotobacter chroococcum	0	3.0	1.5	6,000	5,000	3,000
Pseudomonas fluorescens						
Strain 1	200,000	100,000	100	1,000	1,000	1,000,000
Strain 2	1,000	30,000	100,000	1,000,000	100,000	1,000

TABLE 16-4

Percentage of Soil, Rhizosphere, and Rhizoplane Bacteria from Barley Roots in Three Nutritional Groups (*28*)

Nutritional group[a]	Control soil	Rhizosphere	Rhizoplane
I (inorganic salts, glucose)	3.8	4.0	6.0
II (inorganic salts, glucose, amino acids)	12.6	24.0	32.9
VII (inorganic salts, glucose, yeast, soil extracts)	83.6	70.0	59.7

[a] For the definition of "nutritional group" see Lochhead and Chase (*29*).

identical growth rates and reached over 10^9 cells per milliliter when grown separately in soybean-root extract medium but when they were grown together, *Arthrobacter* reached values between 10^7 to 10^8, and *Pseudomonas* almost 10^{10}, cells per milliliter. This is clearly a case in which neither growth rate nor nutritional requirements but, rather, antagonism between organisms is responsible for the selective stimulation given by the root extract, and undoubtedly it occurs in the natural rhizosphere.

One of the most consistent differences between bacteria isolated from the rhizosphere and those from other soil is in nutritional requirement. Lochhead, Katznelson, and co-workers have shown that a larger proportion of rhizosphere isolates than of soil cultures requires amino acids for maximal growth; some of their results are given in Table 16-4.

Replying to criticism (*30*) of the validity of the nutritional classifications used for demonstrating differences between soil and rhizosphere isolates, Lochhead and Rouatt (*31*) summarized fifteen years of experiments covering nine plant species (several of different ages) in twenty-three trials, to show that without exception the proportions of bacteria requiring amino acids were higher in the rhizosphere than in the control soil.

A further difference in the nutritional requirements of soil and rhizosphere bacteria was revealed in the ratios of direct counts to plate counts, reported by Louw and Webley (*8*). The fact that only about 10% of soil bacteria grew on a "nonselective" agar while up to 72% of rhizosphere bacteria grew on the same medium probably reflects the more fastidious nutritional requirements of the former. Further, it means that, providing the majority of soil bacteria are viable, their nutritional requirements are in actual fact considerably more different from those of rhizosphere bacteria than is indicated by tests on cultures isolated on agar media.

Besides the differences in nutritional requirement there are differences in physiological activity. In general, rhizosphere bacteria are more responsive to readily available nutrients. Zagallo and Katznelson (*32*) have shown that the average oxygen uptake of rhizosphere isolates supplied with various substrates, such as sucrose, glucose, acetate, succinate, and alanine, was greater than that of soil isolates supplied with similar substrates. However, there is considerable overlap in the activities of the individual members of the two groups. This indicates that both soil and rhizosphere populations isolated on agar media cover a similar range of organisms but that the rhizosphere sample is richer in bacteria capable of responding to nutrients such as glucose and alanine. If the population of the rhizoplane had been examined, rather than that of the rhizosphere, there would have been a higher proportion of responsive bacteria with less overlapping by the more active soil bacteria.

In a study of the effects of root exudate added to media of different complexities on bacteria isolated from the rhizosphere of young pea plants and from corresponding control soil, Rovira (*33*) showed that the larger mean response was made by the rhizosphere isolates. The overlap in the responses of the two sets was similar to that found by Zagallo and Katznelson. In general, isolates from the rhizosphere exhibited a more prolific growth than did those from control soil in similar media; for example, in a liquid medium of mineral salts plus 0.1 % glucose 6 of the 19 soil isolates showed dense turbidity, compared with 16 of the 19 rhizosphere isolates.

The ability of almost three hundred bacterial cultures isolated from soil and the rhizospheres of wheat and subterranean clover to colonize the roots of wheat and clover growing in sterile sand was studied by Rovira and Brisbane (*34*). They found that there was a high correlation between the ability of pure cultures to colonize densely the rhizosphere and the following characteristics: Gram negativity, pleomorphism, branching rods, rapid growth on glucose, response to supplements of amino acids, production of ammonia from peptone, production of acid from glucose, positive response to the arginine test, sensitivity to chloramphenicol, and resistance to erythromycin and penicillin.

2. *Fungi*

There is ample evidence that the spores of many nonpathogenic fungal species lying dormant in soil are stimulated to germinate by the proximity of plant roots (*35,36*). Schroth and Hildebrand (*37*) have cited several reports that the germination of fungal spores in the rhizosphere is non-specific. However, Buxton (*38*) showed that exudate from wilt-resistant peas inhibited the germination of spores of *Fusarium oxysporum* Fr. f. *pisi* but that exudate from susceptible peas promoted it.

TABLE 16-5

Percentage Frequency of Occurrence of Some Fungi from Washed Bean Roots, Bean Rhizosphere, and Control Soil (*39*)

Fungus	Roots		Rhizosphere soil[b]		Control soil	
	40 days	190 days[a]	Soil-washing techn.	Dilution-plate techn.	Soil-washing techn.	Dilution-plate techn.
Mortierella spp.	9	16	34	18	35	20
Mucor spp.	0	0	20	1	2	1
Cylindrocarpon radicola	5	39	0		2	1
Fusarium spp.	55	16	21	5	2	2
Gliocladium spp.	11	7		6	0	7
Penicillium spp.	16	2	7	55	7	44
Trichoderma viride	4	0	8	1	9	3
Sterile mycelia	0	9	4	2	3	1
Other species	0	11	6	12	40	21

[a] Moribund roots.

[b] Rhizosphere soil from mature dwarf bean plants.

The results of Parkinson (*39*) given in Table 16-5 clearly demonstrate the influence of isolation technique on the range of fungal species from control soil and bean rhizosphere. The dominant genus found in both samples by the dilution-plate technique was *Penicillium*, but the dominant genera found by the soil-washing technique (for isolating hyphae) were *Mortierella* in the soil and *Mortierella*, *Mucor*, and *Fusarium* in the rhizosphere. On the washed roots of 40-day-old plants *Fusarium* constituted more than half the isolates, whereas on moribund roots *Cylindrocarpon radicicola* was dominant.

Parkinson et al. (*10*) have shown a succession of fungi with age of root; not only do they become more abundant in the older parts of the root, but the species change. They found that as the root grows through soil there is a successive lateral colonization of the root from the soil; the root tip is almost devoid of fungi, the zone behind the root tip contains the "casual" colonizers, and the older part of the root supports more specialized fungi, including *Fusarium oxysporum*, *Cylindrocarpon radicicola*, *Gliocladium* spp., *Penicillium* spp., *Trichoderma viride*, and sterile dark forms.

C. Extent of the Rhizosphere

In any assessment of the importance of the rhizosphere in the metabolism of soil under crop or pasture it is essential to know the dimensions

of the rhizosphere. This is difficult to know, because the rhizosphere is not a uniform zone, and its extent will be affected by the plant species, soil type, soil moisture content, particular portion of the root system, and other factors. Clark (*40*) presented the following results for cotton: root-surface scrapings, 51×10^6 bacteria per gram; soil within 1 to 5 mm of roots, 13×10^6 bacteria per gram; soil 5 mm beyond the roots, 5.5×10^6 bacteria per gram.

In a study with clover and paspalum grown in narrow boxes, from which one side could be removed and soil sampled at different distances from the roots, it was shown that the rhizosphere, as assessed by bacterial plate counts on a nonselective medium, is restricted to within 1 to 2 mm from the root (*41*). Soil which was shaken from the roots gave no higher counts than soil sampled 5 to 10 mm or 10 to 20 mm from the root; in comparison, the roots, together with the firmly adhering soil, gave high R/S ratios. No attempt was made to distinguish rhizoplane and rhizosphere populations, so estimates of numbers in the firmly adhering soil were higher than if the rhizoplane had been excluded.

Papavizas and Davey (*42*) used an ingenious multiple-tube device for sampling 3 mm cores at distances of 0 to 22 cm from blue lupin roots. Blue lupin was selected because of its sturdy tap root and the predictable directions taken by its secondary and lateral roots during the seedling stages. Thus it was possible to sample in the rhizosphere zone at known distances from the main root without interference from lateral roots. The results (Table 16-6) indicate that the rhizosphere zone extended for at least 18 mm from the root; however, inadequate replication and absence of any indication of the statistical significance of the results detracts from the value of the findings. There seems little doubt of the significance of the differences between populations of rhizoplane and of control soil, but the increase of twofold or less between other zones and control soil are within the variation obtained for replicate soil samples. An examination of some of the qualitative differences in fungi shows that the extent of the rhizosphere varied with species; for example, *Cylindrocarpon radicicola* occurred only in the rhizoplane, *Paecilomyces marquandii* had the greatest numbers in the rhizoplane and was not found 12 mm beyond the root, and *Aspergillus ustus* was most numerous in the rhizoplane, falling off with distance from the root but even at 18 mm being twice as abundant as in the control soil. The *Fusarium oxysporum* and *Trichoderma viride* counts were not affected by the root. It is obvious from this that general bacterial and fungal counts may not be sufficiently sensitive to define the zone of root influence, and an investigation of certain species may yield more valuable results.

Wallace (*43*) showed that in wet sand at 15 cm suction eelworm cysts were stimulated to hatch 3 cm distant from plant roots, while larvae could

TABLE 16-6

Extent of the Rhizosphere of 18-Day-Old Blue Lupin Seedlings (*42*)

Distance from root mm	Microorganisms, thousands per gram oven-dried soil			Fungi, number per gram oven-dried soil		
	Bacteria	Strepto-mycetes	Fungi	Aspergillus ustus	Cylindro-carpon radicicola	Paecilo-myces marquandii
0[a]	159,000	46,700	355	5,650	4,940	9,000
0–3	49,000	15,500	176	3,360	0	2,800
3–6	38,000	11,400	170	2,920	0	1,600
9–12	37,400	11,800	130	2,880	0	1,500
15–18	34,170	10,100	117	2,270	0	0
80[b]	27,300	9,100	91	1,000	0	0

[a] Rhizoplane. [b] Control soil.

be attracted as much as 4.5 cm distant. The specificity of eelworms in their response to stimulants makes them an effective tool for measuring the extent of the rhizosphere, but it should be remembered that at lower water contents or in the presence of sorbing clays, as in normal soil, the zones of influence could not be expected to be as sensitive. Barley (*44*) has calculated that 25% of the bulk volume of the top 15 cm of soil under an oat crop at the dough stage lies within 0.1 mm of the root, illustrating that even under cereal a very substantial proportion of the soil must be regarded as rhizosphere. Under a grass sward a greater proportion of the soil would be influenced by plant roots and, as Barley and Sedgley (*45*) calculated, at a depth of 2 cm under an Italian rye-grass sward the mean distance between lateral roots was 3 mm on a horizontal plane.

D. Effect of Plant Age on Rhizosphere Microflora

Bacterial colonies can be seen developing in the zone of elongation of roots within hours of the root's emergence from the seed (*14*). Initially this colonization takes the form of widely separated small clusters of cells but, as the root ages, the colonies increase in size and eventually merge to form almost a complete mantle over the root. The rhizosphere population expressed on a soil-weight basis or as the R/S ratio continues to increase on most plants up to the peak of physiological activity. This is shown in Table 16-7, in which some of the results of Rivière (*46*) with wheat are summarized.

TABLE 16-7

R/S Ratios of Bacteria, Actinomycetes, and Fungi in the Rhizosphere of Wheat at Four Stages of Development (*46*)

	R/S ratio[a]			
	Seedling (8 days)	Tillering	Flowering	Maturity
Bacteria	3.1	27.7	16.8	5.4
Actinomycetes	1.9	35.1	16.7	1.5
Fungi	1.0	274	8.9	10.7

[a] Counts estimated by the dilution-plate method.

In the colonization of the root surface the fungi generally lag behind the bacteria, and the results in Table 16-8 show that the amount of mycelia on the roots increases with age of the roots in three plant species.

TABLE 16-8

Frequency of Occurrence of Fungal Hyphae on Roots of 1-, 2-, 4- and 10-Day-Old Plants, Recorded by Direct Observation (*10*)

	Frequency of occurrence[a]					
				10 days		
	1 day	2 days	4 days	Tip	Mid	Crown
Dwarf bean	0	12	45	12	72	75
Barley	0	9	22	33	66	67
Cabbage	0	15	8	5	70	99

[a] Expressed as the percentage of 2-mm segments of washed roots which showed hyphae.

16-5. FACTORS RESPONSIBLE FOR THE RHIZOSPHERE EFFECT

Starkey (*47*) suggested that the selective stimulation of microorganisms by plant roots was due to the following factors:

Sloughed-off root cell debris.
Release of soluble organic compounds by roots.
Higher concentration of carbon dioxide.
Lower concentration of oxygen.
Lower concentrations of nutrient ions.
Partial desiccation of soil due to the absorption of water by roots.

Although all these factors and, possibly, others play a part in the nutrition of rhizosphere organisms, the major factors are the debris of root-cap cells, moribund root hair and cortical cells, and soluble organic materials released or exuded by intact roots.

A. Root Cell Debris

As roots move through soil the root-cap cells slough off into the surrounding soil, and this material will be in part responsible for providing nutrients to soil microorganisms near the root tip and elongating zone. Root hairs become moribund as the root ages, and in direct microscopy they appear densely colonized by bacteria (*48*). In the older root regions cortical cells also become moribund and provide foodstuff for fungi and bacteria. Roots of permanent pasture plants die and undergo decomposition after cutting or grazing of the foliage (*49*), and this material will greatly stimulate microbial metabolism in soil although, strictly speaking, such stimulation cannot be regarded as a rhizosphere effect.

The contribution which root cell detritus makes to the nutrition and selective stimulation of microorganisms in natural soil is difficult to assess. Rovira (*50*) reported that the roots of 50 pea plants growing in quartz sand released 16.7 and 31.5 mg of cell debris over 10 and 21 days, respectively, and the roots of 50 oat plants released 6.6 and 14.8 mg over the same periods. Although this amounted to less than the quantities of soluble organic materials released during the same periods, it was still quite substantial as substrate for microorganisms, and especially so, considering that the methods used in these experiments probably underestimated the quantities of cell debris.

B. Root Exudates

There is now much evidence that intact, healthy plant roots exude sufficient organic materials to support large populations of microorganisms (*26,37,48,51*). The studies, which are reported in more than one hundred and twenty publications and have been conducted with many different plant species, reveal the following compounds in exudates from intact plant roots: 10 sugars, 21 amino acids, 10 vitamins, 11 organic acids, 4 nucleotides, 11 miscellaneous compounds, 16 fungal stimulators or attractors, 3 fungal inhibitors, 4 eelworm-hatching factors, and 2 eelworm attractors.

An indication of the range of compounds exuded by a single plant species is obtained by preparing a composite table of all the compounds exuded from wheat roots, as reported by ten different workers; see Table 16-9.

TABLE 16-9

Compounds Reported in Wheat Root Exudate

Sugars	Amino acids	Organic acids	Nucleotides, flavonones	Enzymes
Glucose	Leucine and isoleucine	Oxalic	Flavonone	Invertase
Fructose	Valine	Malic	Adenine	Amylase
Maltose	γ-Amino butyric acid	Acetic	Guanine	Protease
Galactose	Glutamine	Propionic		
Ribose	α-Alanine	Butyric		
Xylose	β-Alanine	Valeric		
Rhamnose	Asparagine	Citric		
Arabinose	Serine	Succinic		
Raffinose	Glutamic acid	Fumaric		
Oligosaccharides	Aspartic acid	Glycolic		
	Glycine			
	Phenylalanine			
	Threonine			
	Tyrosine			
	Lysine			
	Proline			
	Methionine			
	Cystathionine			

Composite tables are of little value in any attempt at assessing either the exudation from a particular plant species under a given set of conditions or the differences between the exudates. Comparisons between plants are valid only if conducted in identical environments and at comparable stages of plant development. Experiments with plants of similar ages grown under identical conditions show that plants do differ considerably in both quantity and type of compound exuded from their roots (*48,52*). This may be illustrated by comparing the patterns of the major amino acids in the exudates of roots of peas and oats, Table 16-10.

Vancura and Hovadik (*52*) compared the exudation of amino acids, organic acids, and sugars from six different plants. The general conclusion which may be drawn from their results is that wheat and barley root exudates show reasonably similar patterns that differ from those of tomato and red pepper exudates, which are themselves similar. Cucumber, turnip, and cabbage each appear to have a distinctive pattern. Even though wheat and barley are generally similar, there are differences between them; for example, γ-amino butyric acid occurs in wheat but not barley exudate.

TABLE 16-10

Amino Acids in Exudates from the Roots of Peas and Oats during 21 Days (*50*)

	Intensity of spot[a]	
	Peas, (three)[b]	Oats, (twenty)[b]
Asparagine	3	2
Aspartic acid	2	1
Serine	3	3
Glutamine	4	1
Homoserine	4	0
α-Alanine	4	1
Leucine	1	2
Lysine	2	3
Threonine	4	Trace
Glycine	0	3

[a] Spots on chromatogram rated 1 to 5 according to intensity.

[b] Number of plants required per chromatogram.

These differences are probably sufficient to cause qualitative and quantitative differences in the rhizoplane and rhizosphere microflora.

Although there is ample evidence that a wide range of compounds is exuded from intact plant roots, it is essential to have some knowledge of the quantities of soluble organic materials in the exudates. The actual amount of nutrient exuded from roots tends to determine the number of microorganisms which develop in the rhizosphere, whereas the qualitative differences determine their specificities.

Meshkov (*53*) reported that in two experiments peas growing for 20 days in sterile nutrient solution exuded 2.9 and 4.3 mg of reducing sugars per plant, but that corn exuded 8.4 and 8.2 mg in corresponding experiments. This represents 0.14 to 0.23% of the plant dry weight for peas and 0.23 to 0.35% for the corn. Rovira (*50*) found that pea plants growing in sterilized quartz sand exuded 1.1 and 1.4 mg of soluble material per plant during 10 and 21 days, respectively. During the same periods oats exuded 0.5 and 0.6 mg per plant.

Krasil'nikov (*26*) calculated the amount of energy material required to support the bacteria and yeast in a nutrient culture solution in which wheat was growing for 15 days; the roots of each plant released about 17 mg of organic substances utilizable by bacteria and 7 mg utilizable by yeasts.

Rivière (*46*) made direct measurements of the quantities of organic acids exuded by sterile wheat roots which, taken together with measurements of the amino acids and sugars, indicate that Krasil'nikov's indirect assessment is not extreme. Rivière found that a single wheat plant grown under sterile conditions in nutrient culture solution until the tillering stage exuded 13 mg of acetic, 3.5 mg of propionic, 2 mg of butyric, and 1.5 mg of valeric acid.

Most estimates of the amounts of material exuded by roots have been conducted with culture solutions, and for this reason the study made by Harmsen and Jager (*54*), in which sand-and-clay mixtures were used, are particularly valuable. These investigators packed synthetic soil of 70% sand, 25% fine potassium feldspar, and 5% kaolinite into narrow, perforated metal cylinders in which they planted wheat, spinach, or vetch. They placed the cylinders in large pots containing 600 g of the same synthetic soil. After 6 to 8 weeks they removed the cylinders and washed the soil within them to collect water-soluble exudates, washing first the soil from a zone 1 cm thick surrounding the cylinder and then the remaining soil. Carbon and nitrogen analyses of these washings showed a definite concentration gradient from the root chamber outward. The carbon results (which are more reliable than the nitrogen results because of the nitrogen added in the plant-nutrient solution) are presented in Table 16-11.

These results show a substantial increase in water-soluble organic matter in the rhizosphere, especially with wheat in the presence of added feldspar and kaolinite, but no figures for total carbon are given except with vetch. With vetch the amount of carbon exuded ranges from 1.6 to 2.9% of the carbon in the roots. Since the weight of the soil within the cylinder is not given, it is not possible to assess accurately the total exudation from wheat and spinach, but approximate calculations based on the vetch results indicate that the cylinder held between 30 and 35 g of soil and that the 1 cm zone around the cylinder consisted of approximately 50 g of soil. On this basis one may calculate that during 2 months of growth wheat exudes from 2.6 to 22.5 mg of carbon per plant. This is a minimal figure, because no allowance can be made for the amounts of exudate decomposed and assimilated by the microflora.

It requires a considerable refinement of technique to obtain absolute figures on the amounts of organic matter exuded in nonsterile systems, because it is necessary to measure both the carbon in microbial cells and

TABLE 16-11

Exudation of Carbon from the Roots of Wheat, Spinach, and Vetch[a] (*54*)

Plant	Carbon exuded per 5 g of dry soil,[b] mg			Total carbon, mg	Carbon exuded per 100 mg of dry root, mg
	A	B	C		
Vetch	1.6			11.8	2.9
	0.7	0.1	0.2	7.0	1.6
	0.6[c]			3.6	2.2
	0.7[c]		0.1	5.4	2.6
Wheat	1.7				
	8.7[d]	1.4	0.5		
	2.7	0.1			
Spinach	2.1[d]	0.1			
	2.6				

[a] Vetch was grown for 6 weeks, wheat and spinach for 8 weeks; all were grown four plants to a pot.

[b] A, soil from cylinder; B, soil from 1 cm zone around cylinder; C, soil outside zone B.

[c] Sterile cultures.

[d] Soil with more than 20% feldspar and 5% kaolinite.

the carbon in carbon dioxide lost from the soil by both sterile and nonsterile root systems. Therefore, the figures given by Harmsen and Jager for the exudation of nonsterile vetch are probably far too low. Even if only half the exuded carbon in the nonsterile system were metabolized by microorganisms (this is a very conservative estimate, considering the major components of sterile exudate, viz. sugars, amino acids, and organic acids), one could calculate that the amount of exudate from nonsterile roots may be four times that from sterile roots.

These results raise one of the most important questions concerning current rhizosphere and root exudate investigations: is it valid to predict the significance of root exudates in relation to rhizosphere microflora from results given by plants grown under sterile conditions? Root exudate patterns may be changed by microorganisms in several ways, such as by altering the permeability of root cells, modifying root metabolism, and assimilating and altering many of the substances exuded by roots. Norman (*55*) recorded that polymyxin, the antibiotic produced by *Bacillus polymyxa*, increased the leakage of amino acids from roots, and it is quite conceivable

that other substances associated with the development of colonies of certain bacteria upon roots would promote exudation in the immediate vicinity of the colonies. Martin (*56*) measured the action of filtrates of several bacterial cultures on the release of scopoletin from sterile oat roots and found that several increased the release threefold while others had no effect. The use of culture filtrates introduces a very artificial element into the experiment, seeing that it is difficult, if not impossible, to equate the concentrations of active components in filtrates with those synthesized in situ by the rhizoplane microflora. However, Martin's results again illustrate the importance of considering the role of microorganisms in the root exudation processes.

Factors other than microorganisms have been shown to influence the amounts and types of material which exudes from plant roots. High light intensities and temperatures both favor exudation (*57*), as does the temporary wilting of the plants (*58*). Exudation from a single plant species also varies, according to the stage of development of the plant (*46,50,52*).

Considerable caution is required in assessing the significance of any root exudate finding in relation to the selective stimulation of soil microorganisms by plant roots because of the lack of control of the above-mentioned factors.

A further point to be considered in relation to root exudates is their relative concentrations in the rhizosphere. The most active region of the root with regard to exudation appears to be the zone of elongation behind the root cap, which has been assessed both chemically (*59,60*) and by the attraction of motile zoospores (*61*) and nematodes (*62,63*). If this is the case, then the greatest microbial activity would be here. However, Frenzel (*64*) used nutritional mutants of *Neurospora* to show that different amino acids were exuded from different regions along the root of *Helianthus annuus* L: threonine and asparagine from the root tip; and leucine, valine, glutamic acid, and phenylalanine from the root-hair zone.

The release of nitrogen from the roots of nodulated legumes by exudation and from senescent nodules is an important matter affecting the rhizosphere of legumes, but it is not considered within the scope of this review; aspects of the problem are covered in the literature (*26,49,65–68*).

16-6. USE OF ^{14}C IN RHIZOSPHERE STUDIES

The individual components of root exudates occur in minute amounts, and this feature introduces special experimental difficulties into the identification and assay of them. The finer sensitivity of tracer techniques offers a solution of the problem. Plants may be easily labeled with ^{14}C by allowing

photosynthesis to proceed in an atmosphere containing ^{14}C-carbon dioxide. This method has been used widely in studies of translocation in plants and has also been used by several laboratories for studying the exudation of organic compounds from roots (*69,70–72*).

Subba Rao et al. (*69*) labeled tomato plants by this means and, extracting the soil around the plants, traced the liberation of radioactivity from the roots into the surrounding soil. The radioactivity decreased with increasing distance from the root. Part of it could be extracted from the soil only after acid hydrolysis; this fraction apparently represented exuded compounds which had been fixed by soil microorganisms. Similar studies were conducted by Ivanov et al. (*70*), who applied ^{14}C-carbon dioxide atmosphere to the tops of maize and beans in soil and demonstrated that the radioactivity was transferred to neighboring plants.

Slankis and co-workers (*71*) over a period of 8 days collected exudates from the sterile root systems of white pine seedlings grown in nutrient solution. Fractionation of the exudate by paper chromatography and ionophoresis revealed more than thirty-five radioactive compounds; a number of these were identified by cochromatography and coionophoresis with known, unlabeled substances.

We have labeled plants by the method of assimilation of ^{14}C–carbon dioxide in some studies of the exudation from roots of wheat seedlings (*72*). Before labeling, the seedlings were grown aseptically on fiber-glass mesh in a test tube containing mineral solution. They were then removed from the tube without damaging or contaminating the roots, and the mesh was cut away. The plants were then transferred to an apparatus (Fig. 16-7), which was so designed that the foliage alone could be exposed to the atmosphere containing the ^{14}C-carbon dioxide; provision was made for aerating the solution surrounding the roots. The plants were first exposed, in the light, to the ^{14}C-carbon dioxide for 1 hour and then exposed to atmospheric carbon dioxide. The solution surrounding the roots was examined for nonvolatile exudates, and these were found to represent 25% of the label in the solution collected without aeration. Radioactive substances first appeared in the exudate 3 hours after labeling and were still emerging after 24 hours. During this period 0.1 to 0.4% of the assimilated ^{14}C was exuded. Significantly more radioactivity (about 1.6 times more) appeared in the exudates collected in distilled water than in those collected in mineral nutrient solution; this effect may have been due to a rupturing of the root hairs by the distilled water, such as has been observed by Roberts (*73*). The exudation of radioactivity was slightly greater when the solution surrounding the roots was bubbled with air than when it was bubbled with nitrogen. These experiments illustrate that changes in root environment can affect the amount and, perhaps, the pattern of exudation.

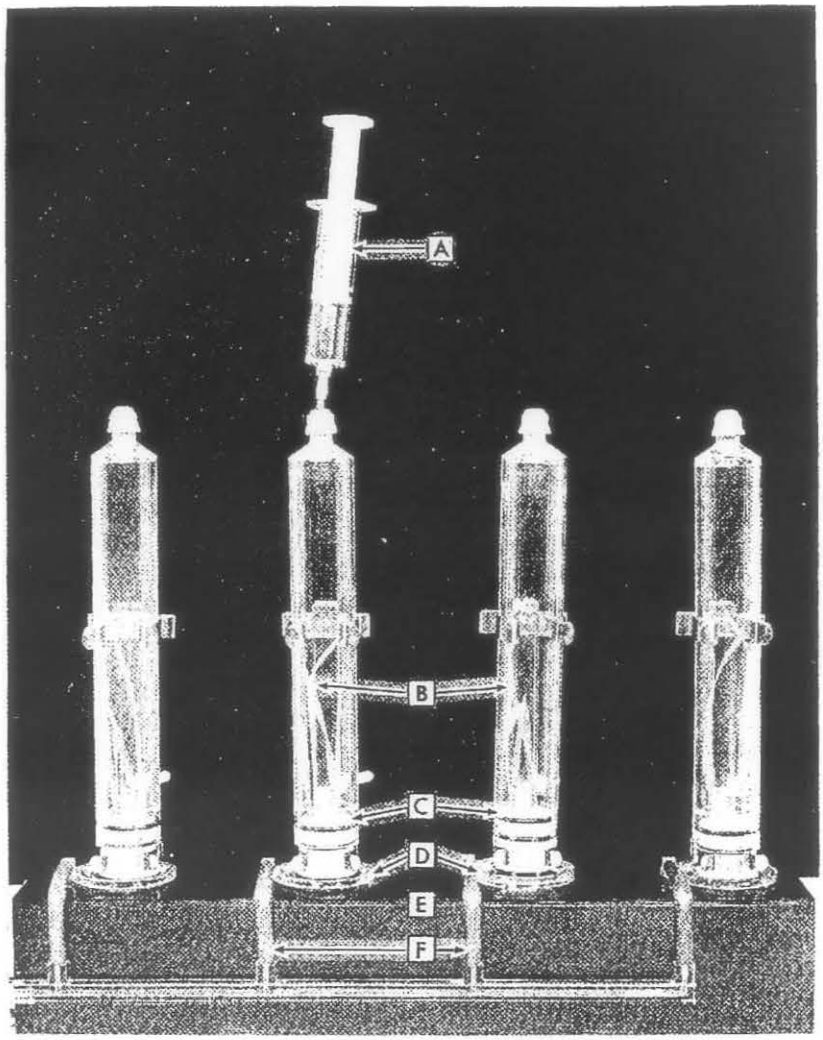

Fig. 16-7. Apparatus for administration of $^{14}CO_2$ to tops of wheat plants: (A) syringe containing $^{14}CO_2$-enriched air; (B) foliage enclosed in plastic tube; (C) split plastic stopper with grooves for stems of plants and O-ring seals with outer tube; (D) beaker containing roots with provision for aeration of solution; (E) black plastic outer container with temperature control; (F) air line.

The exudates were fractionated on ion-exchange resins and by paper chromatography and examined by radioautography or radio-strip-scanning for radioactive spots. Cochromatographic identification of the components should be possible with several solvent systems and known, unlabeled compounds.

There are several advantages in the experimental design used in the work reported here. With seedlings it is difficult to discriminate between true root exudates and substances leaking through the seed coat. Foliar labeling overcame this difficulty, for the spatial separation of the top of the plant from the roots guards against contamination of the root with foliar exudate.

The use of ^{14}C labeling of photosynthate in studies of root exudates offers the following advantages:

1. It ensures that all the major components in the exudate are detected, whereas in normal chemical and colorimetric tests the reagents determine the type of compound detected.
2. It is sufficiently sensitive for the study of exudate from individual plants collected over short periods of time.
3. The measurement of radioactivity could yield data on the comparative composition of exudate, providing the plant is so labeled that the components of at least the soluble fraction are of equal specific activity. Such plants could be obtained by exposing them to ^{14}C-carbon dioxide for longer times than those used by the workers in the preliminary studies discussed here. It should then be possible to study the effects of various conditions on exudate patterns.

Labeling by foliar-application techniques was used in studies of the exudation of exogenous compounds with growth-modifying properties, such as α-methoxyphenylacetic acid (*74*) and related substances (*75*). α-Methoxyphenylacetic acid labeled with ^{14}C was applied to the leaves of bean plants, some growing in tap water and others in nutrient solution. The acid was translocated to the roots and exuded chemically unchanged. Radioactivity was detected in the solution surrounding the roots approximately 5 hours after foliar application. By measuring this radioactivity it was possible to study the exudation and reabsorption of the acid with respect to different conditions, such as aeration of the solution around the roots, removal of portions of the root system, and variations in the amount of acid applied to the leaves.

For exudation studies of endogenous compounds the assimilation of the mixture of ^{14}C-carbon dioxide by the whole plant is more satisfactory than foliar application of ^{14}C-labeled compounds because the latter may overload the plant and affect the pattern of exudation.

16-7. MUCILAGINOUS MATERIAL ON ROOTS

A layer of material coating the epidermal surfaces of roots of plants has been observed under light and electron microscopes. The plant roots examined include onion (*76*), radish (*77*), oats (Rovira, unpublished observations), barley (*15,128*), and medic (*16*). Although very little is known of its nature, this substance is usually referred to as "mucilaginous material" or "mucigel" because of its viscous nature. Since this mucigel is present on root systems grown under aseptic conditions (*16*), it must be produced by the plant and not by microorganisms in the rhizosphere although, when present, the latter may contribute similar substances (*78*). Mucigel is described as a matrix which has a granular appearance in electron micrographs (*76*). It may contain oil-like droplets, which stain red with Sudan III (*76*), and cellulose microfibrils in a network (*79*). Little is known of its chemical composition with respect to different plant species; the material covering onion roots (*76*) and radish-root hairs (*77*) have stained faintly with ruthenium red, suggesting that it may contain some pectic substances.

Studies of the composition of the mucigel from sterile oat seedlings are summarized in Table 16-12. The mucigel was insoluble in cold water, but hot water dissolved a small amount of oligosaccharide, which was converted by hydrolysis to a mixture of glucose and fructose. Aqueous alcohol (80%) extracted larger amounts of the oligosaccharide containing glucose and fructose. The detection of this oligosaccharide does not preclude the presence of other constituents, since tests for neutral sugars only were performed.

The layer of mucigel is thick compared with the epidermal cell wall, reaching several microns in thickness. In electron micrographs of legumes prepared by Dart and Mercer (*16*) it seems to be bounded by an outer electron-dense membrane, but Jenny and Grossenbacher (*15*) used colloidal iron hydroxide particles to define its boundary on barley roots. These

TABLE 16-12

Analysis of Mucilaginous Material from Roots of Sterile Oat Seedlings[a]

Root washings	Carbohydrates detected by paper chromatography	Composition of oligosaccharide
Cold water	Glucose, fructose, sucrose (trace)	
Hot water	Glucose, fructose, sucrose, oligosaccharide	Glucose, fructose
Alcohol, 80%	Glucose, fructose, oligosaccharide	Glucose, fructose

[a] The authors are indebted to the late Dr. R. B. Duff, Macaulay Institute of Soil Research, Aberdeen, Scotland, for conducting the analyses.

boundaries may be real and due to different constituents of the mucigel or they may be artifacts of different preparative procedures.

Barley roots grown in clay or sand when fixed and embedded in situ gave electron micrographs which showed very intimate contact between the mucigel and the mineral particles (*15*). Jenny and Grossenbacher (*15*) suggested that, since the contact is so close, chemical bonding occurs between carboxyl groups in the mucigel and the positively charged edge surfaces or exchangeable ions of clays. Consequently, in the presence of hydrogen ions, which are produced by metabolic reactions and diffuse from the roots, the mucigel mobilizes cations from minerals by contact exchange. These cations diffuse toward the root along a chain of exchange sites in the mucigel by cation-exchange diffusion and so can be transferred at a much faster rate than by diffusion in solution (*80*).

The region of the root which produces the mucigel is not known. Dart and Mercer (*16*) noted that the mucigel appears most prominently near the root-cap region and suggested that the two may be continuous. They consider, however, that it is formed by secretion from epidermal cells. This theory agreed with that of Scott et al. (*76*), who suggested that liquid precursors of mucilage exude through plasmodesmata in the epidermal cell walls. In support of this, they noted that the faces of epidermal cells were pitted; however, because no canals in the cuticle through which mucilage might exude were visible, they suggested that such may have been obscured by shrinkage of the membrane during preparation. Drawings and photographs by Esau (*81*) show roots of *Zea mays* and *Nicotiana tabacum* with mucilaginous material extending over the surface of the root in the region of the root cap. Esau (*81*) states that this material originates through gelatinization of the wall between the root cap and the protoderm. It could be assumed that, as the root elongates, this gelatinized material stays associated with the epidermal surface and hence the root receives a covering of mucigel, which may or may not be supplemented by further production of mucilage.

Rhizobia are packed in a layer just under the mucigel membrane on legume roots with mucigel between the zone of bacteria and the epidermal cell wall (*16*). Where rhizobia have become embedded in the mucigel zone they appear to undergo lysis. Photographs of the barley-root mucigel (*15*) show that it also is invaded by bacteria. In both cases the mucigel may be a substrate for the bacteria, or they may be simply embedded in the mucilaginous material without degrading and absorbing it. Dart and Mercer (*16*) observed, however, that rhizobia in the mucigel contained inclusion granules, possibly poly-β-hydroxybutyric acid, which is formed when the culture medium is rich in carbohydrate. If the mucigel is a pectic acid polymer or a polysaccharide, it seems reasonable that some microorganisms

might utilize it as a carbon source. Its presence on nonsterile roots, however, indicates that utilization by microorganisms is limited or that there is continuous production of mucigel along the length of the root rather than only in the root-cap region, as previously discussed.

The mucigel could entrap substances of high molecular weight released by excretion or lysis from root cells or from rhizosphere bacteria. One interesting possibility is the presence of enzymes in mucigel. Nuclease and glycerophosphatase activity in the gelatinous material surrounding the root tips of corn is reported by Rogers et al. (*82,83*). Although the authors claim that these enzymes are of plant origin, they may have originated from associated microorganisms, since the plants were grown under nonsterile conditions. Estermann and McLaren (*128*) have shown that phosphatase originates from both sources in barley mucigel. Rogers and his co-workers destroyed the viability of microorganisms in the mucigel by dipping the roots in mercuric chloride before immersing them in nucleic acid or glycerophosphate; however, this treatment does not necessarily inactivate all the microbial enzymes.

The presence of a mucigel layer on the roots of some plants (whether it exists on all plants is unknown) could modify the environment of the rhizosphere in the following ways: by extending the ion-exchange zone of the root, by providing a possible source of carbohydrate material for the nutrition of microorganisms in the rhizosphere, and by entrapping enzymes derived from plant or microbial sources.

16-8. EXTRACELLULAR ENZYMES FROM PLANT ROOTS AND MICROORGANISMS

Extracellular enzymes have been reported in the vicinity of roots, but it is not always clear whether they originate from plant cells or microorganisms.

A. Extracellular Enzymes from Plant Roots

The association of extracellular enzymes with roots grown under aseptic conditions has been recorded in several cases. The classification of enzymes as extracellular is usually based on two criteria: (a) the enzymes are released into the medium surrounding the root, and (b) the enzymes are bound to the external surfaces of the root cells and utilize substrates which cannot penetrate these cells. In most cases, however, the necessary evidence for establishing their extracellular origin, as distinguished from their release through cellular lysis, is not provided.

The most critical approach to the problem has been made by Chang and Bandurski (*84*). They studied roots of *Zea mays* grown under aseptic conditions and demonstrated that invertase and nuclease were released from the roots as soluble enzymes, whereas the hydrolysis of cellulose, adenosine

triphosphate, and pyrophosphate was catalyzed only in the presence of the roots. They further demonstrated that there was no protease activity associated with the intact roots and, on the assumption that such would be present if the cytoplasmic contents of root cells had been released, concluded that the enzymes were extracellular in origin. Their evidence would have been stronger if they had tested for protease activity in homogenates of root cells under the same conditions, since, even if present, protease may have been inactivated.

The invertase of corn radicle was considered by Hellebust and Forward (*85*) to be external to the permeability barrier of the cells, because the activities of intact cells and of homogenates were equivalent. They considered that cells of higher plants were normally resistant to sucrose penetration, although they did not test this under the conditions of their experiments.

Another report of extracellular root enzymes is that of Krasil'nikov (*26*), who found that amylase activity was associated with sterile roots of wheat, corn, and peas and that amylase existed in water in which the wheat roots had been immersed. There is also the report of Rogers et al. (*82,83*). that nucleases and phosphatases are associated with the mucigel and sloughed-off cells; this has been discussed in Section 16-7.

Ulrich et al. (*86*) demonstrated that tomato plants growing in soil or sand sterilized by irradiation could utilize inactivated lysozyme as a source of nitrogen. The lysozyme may have been hydrolyzed by the action of extracellular or surface-bound proteases of the root, and the amino acids so released then absorbed by the plant.

It is possible that the enzymes which are reported as extracellular to the root cells may occur naturally as such; on the other hand, they may be initially intracellular enzymes which are released through the lysis of root cells into solutions where they either stay or become sorbed onto the surface of root tissues. The enzymes may also be carried by sloughed-off cells from the root tissues into the surrounding medium, where they may appear to be in solution. Whatever the origin of these enzymes, their presence in the rhizosphere could influence both plant and microbial nutrition. Enzymes such as amylase, cellulase, proteases, lipases, and phosphatases would hydrolyze the high-molecular-weight compounds to lower-weight compounds which could then serve as nutrients for plants or microorganisms in the rhizosphere (*128*). Phosphatase and invertase activity of barley root surfaces can be attributed in great part to enzymes of the root itself, whereas urease activity is entirely a function of rhizoplane organisms (*128*).

An interaction between roots and rhizosphere bacteria in the production of extracellular enzymes by roots was demonstrated by Fåhraeus and Ljunggren (*87*). They reported that, when legumes are inoculated with infective rhizobia, the rhizobia stimulate within the root hair the synthesis of polygalacturonase, some of which is exuded into the surrounding medium. The reaction is quite specific, as only infective rhizobia induced it; clover

rhizobia has no effect on lucerne roots, and vice versa. Highly specific water-soluble, heat-stable, nondialyzable substances which stimulate polygalacturonase production are mainly polysaccharide, but traces of deoxyribonucleic acid may be present (*88*).

B. Extracellular Enzymes from Microorganisms

Extracellular enzyme production by microbial cells has been reviewed by Pollock (*89*) and Davies (*90*). In some cases, such as those of α-amylase and proteinase produced by *Bacillus subtilis*, the extracellular nature of the enzymes is well authenticated, and in many others the evidence is strong (*89*). A number of microorganisms thought to produce such enzymes belong to genera commonly found in soil and rhizosphere; considering this fact and the insoluble nature of various substrates utilized by soil microorganisms, many must produce extracellular enzymes. Because the growth of microorganisms is stimulated in the rhizosphere, an appreciable release of extracellular enzymes from microbial cells might occur in this zone.

16-9. MICROBIAL ACTIVITY IN THE RHIZOSPHERE

In view of the readily available organic matter added to the rhizosphere by plant roots and its consequent effect on the microflora, it is important to have an assessment of the differences in metabolism between rhizosphere and nonrhizosphere soils. One may assess metabolism by measuring respiration, which indicates the general activity of the microflora, or by studying specific processes such as ammonification, nitrification, denitrification, decomposition of organic matter, or phosphate release.

A. Respiration

The contribution which the rhizosphere microorganisms make to the respiration of nonsterile roots was shown by Reuszer (*91*). He found that such roots growing in nutrient solution produced 250% more carbon dioxide than did sterile roots when the results were expressed on a root-weight basis. As Reuszer points out, the microorganisms may affect the root respiration itself, so it is difficult to conclude that the greater evolution of carbon dioxide from the nonsterile system was due to microbial respiration alone. When the respirations of sterile and nonsterile roots growing in vermiculite were compared (the results were expressed as total carbon dioxide evolved per root system rather than calculated back to a root-weight basis), the nonsterile system produced 15.7% more carbon dioxide than the sterile.

Katznelson and Rouatt (*92*) used the Warburg respirometer to measure oxygen uptake of control soil and soil shaken from the roots of several crop

plants which had been grown in the greenhouse and in the field. Greenhouse pea, barley, and clover rhizosphere soil respired twice as fast as the oat or control soil. In the case of the field-grown samples the soils from corn and soybean rhizospheres respired four times as fast as the control; those from barley, rye, and wheat rhizospheres, three times as fast; and those from oat rhizosphere, two times as fast. When substrates such as amino acids were added to these samples, respiration of the rhizosphere soil over an 8-hour period was increased by 100%, but that of the control soil by only 50%. This was consistent with the results of Zagallo and Katznelson (*32*), who used pure cultures of bacteria isolated from control soil and rhizosphere. Katznelson and Rouatt (*92*), on the other hand, found little response from either when a mixture of sugars and their metabolic intermediates was used as the supplement, but the failure of this nonnitrogenous substrate to increase the oxygen uptake greatly probably was because it was supplied at one-tenth the concentration of the amino acids.

In a series of experiments with several soils (*93*) with and without complete fertilizer supplement and planted densely with red clover (*Trifolium pratense*) and paspalum (*Paspalum dilatatum*) it was found that soil from the planted pots which had been sieved free from roots consumed 25 to 50% more oxygen than did the controls. With glucose supplement the

TABLE 16-13

Effect of Clover and Paspalum Roots on Oxygen Uptake of Soil[a] (*93*)

			Oxygen uptake, μl/g/hr		
Fertilizer treatment	Plant	Dry weight (g) roots per 200 g soil	No supplement	Glucose	Compost
None	None		4.2	23.6	18.8
	Clover	0.098	5.2	24.0	23.1
	Paspalum	0.202	5.0	26.0	26.0
Complete (N, P, and K)	None		4.2	24.5	18.5
	Clover	0.366	6.9	24.1	46.6
	Paspalum	1.267	5.2	24.2	31.6

[a] In this experiment the rhizophere soil constituted the complete contents (200 g) of the pot in which the plants had grown. It was passed through a 2 mm sieve to remove visible root fragments, and experiments commenced immediately without any intermediate drying. Supplements in the form of finely ground powder were mixed thoroughly with the soil before placement in Warburg respirometers. The proportions of glucose were 100 mg per 20 g of soil; of compost, 2 g per 20 g of soil.

increase in oxygen uptake was similar in both rhizosphere and control, but with finely ground, sterilized compost prepared from young grass clippings it was considerably more in the rhizosphere. The results for one soil, summarized in Table 16-13, show that when fertilizer was added, clover roots affected the metabolism far more than did paspalum roots, despite the fact that the weight of the former was only a little more than one quarter that of the latter.

One of the problems in conducting experiments of the type described (*92,93*) is to obtain sufficient soil of high activity and representative of the inner rhizosphere. Attempts have been made to produce an artificial rhizosphere with regular additions of root exudate solution (*46,94*). Such techniques have resulted in quantitative and qualitative shifts in the bacterial population very similar to those found in the rhizosphere. Oxygen-uptake experiments with these soils showed no difference between treated and untreated soil, unless glucose was added, and then the rates of the former were 24% higher than those of the latter. No such experiments with amino acid supplements were made, which is unfortunate in view of the reported greater differential response to amino acids made by natural rhizosphere soil than by control soil.

B. Ammonification

Studies of the ammonifying activities of isolates of control soil and rhizosphere bacteria (*28*) have shown R/S ratios greater than 50. One would therefore expect rhizosphere soil to release more ammonia from suitable substrates than control soil; this was shown to be the case by Starkey (*95*), working with soil which supported a wide range of plants. Similarly, one would expect the more rapid decomposition of amino acids in the rhizosphere to release more ammonia; it was shown in studies of soils treated with root exudates that, although they do not release more ammonia from the soil organic matter, they do ammonify and nitrify a peptone supplement more rapidly than untreated soils (*92*).

C. Nitrification

Goring and Clark (*96*) showed that there was less nitrogen available to plants in the rhizosphere than would have been transformed to nitrate if the soil had remained unplanted. This was due, not to any inhibition of nitrification in the rhizosphere but, rather, to an immobilization of nitrogen by the microorganisms in the rhizosphere, and it was demonstrated by a rapid accumulation of mineral nitrogen when the rhizosphere soil was incubated. Estimates of nitrifying bacteria in the rhizosphere (*46,97*) show a definite increase in the numbers of *Nitrosomonas* and *Nitrobacter* in the rhizospheres of wheat, maize, and lucerne. Rivière (*46*) showed that the

number of nitrifiers in wheat rhizosphere increased with the wheat's development, reaching a maximum at tillering. When he added root exudate (*46*), it was only the exudate of 17-day-old plants which significantly increased the number of *Nitrosomonas*, the exudate of younger and older plants having little or no effect. All exudates of plants 17 days old or more had a stimulating effect on *Nitrobacter*.

D. Denitrification

With the very high numbers of denitrifying bacteria in the rhizosphere (*28*) it would be surprising if the losses of nitrogen from this zone were not greater than from control soil. Woldendorp (*98*) demonstrated that 15 to 37% of fertilizer nitrogen was volatilized when applied to permanent grassland below field capacity, the losses from nitrate fertilizer being double those from ammonia fertilizer. He attributed this greater denitrification in the rhizospheres of living plants to three factors: greater numbers of denitrifying bacteria, greater oxygen consumption, and exudation of hydrogen donors by root systems. His finding that oxygen consumption of a permanent-grassland sod containing living roots was 28 times that of an identical sod in which roots had been killed is surprising, but he gives no indication of the means by which the roots were killed. Woldendorp uses his evidence to show that "in soils containing living roots, low oxygen tensions are more commonly encountered than in fallow land of the same soil type." In an attempt to establish the oxygen uptake of the two components of the root–microbe complex Woldendorp measured the oxygen uptake of sterile and nonsterile pea roots growing in vermiculite; he found that it was 50% greater with nonsterile than with sterile roots.

A comparison of the amounts of gaseous denitrification due to perennial rye-grass roots and to pea roots, both under atmospheres of nitrogen, showed that the latter was greater (Table 16-14), and Woldendorp attributes this fact to the peas' greater release of amino acids, which act as hydrogen donors in denitrification. This conclusion is based upon studies of the denitrification of added nitrate by washed cells of *Pseudomonas aeruginosa* with glucose and glutamic acid supplements.

The denitrification curves obtained with *Pseudomonas* (Fig. 16-8), however, differed markedly from those obtained with nonsterile roots (Fig. 16-9), the latter closely resembling bacterial-growth curves; this suggests that the denitrification is due directly to the buildup of a denitrifying population, rather than to the accumulation of hydrogen donors in the rhizosphere.

When living pea roots in vermiculite were placed in Warburg vessels under aerobic conditions with and without added nitrate, and changes in the gas pressures were measured, it was found that denitrification com-

TABLE 16-14

Release of Gaseous Nitrogen Products during Denitrification of 10 mg of Nitrate-Nitrogen from Vermiculite with and without Roots (*98*)

Treatment	Nitrogen gas released, mg
Control (no plants)	0.1
4-day Perennial rye-grass	1.0
14-day Perennial rye-grass	1.7
8-day peas	3.2
21-day peas	3.6

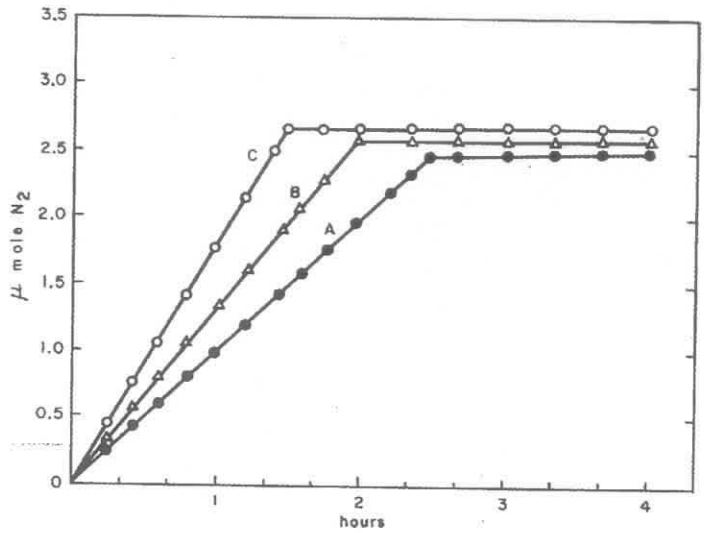

Fig. 16-8. Formation of gaseous reduction products during denitrification by washed cells of *Ps. aeruginosa* in the following substrates: (A) 2.45 μmoles of glucose + 6 μmoles of KNO_3 per milliliter; (B) 4.75 μmoles of glutamic acid + 6μmoles of KNO_3 per milliliter; (C) 2.45 μmoles of glucose + 4.75 μmoles of glutamic acid + 6 μmoles of KNO_3 per milliliter. [From Woldendorp (*98*).]

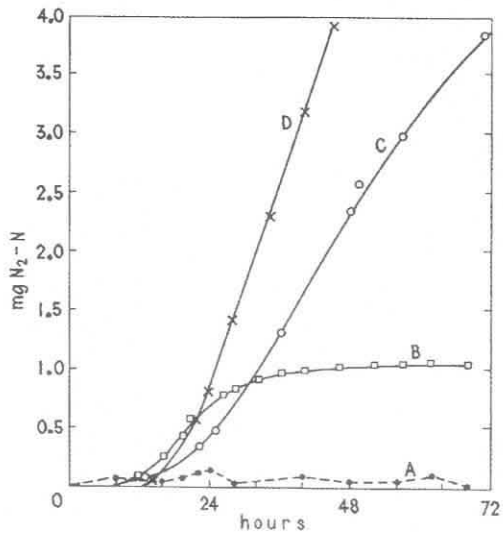

Fig. 16-9. Formation of gaseous reduction products during denitrification of 10 mg of nitrate nitrogen by nonsterile root systems of perennial rye-grass and pea plants: (A) unplanted control; (B) perennial rye-grass, 4 days old; (C) perennial rye-grass, 14 and 28 days old; (D) green peas, 8 days old. [From Woldendorp (*98*).]

menced when the oxygen level within the vessels was reduced to half the atmospheric level. As it would not be uncommon to find such oxygen levels under permanent pasture, these results support the original observations of Woldendorp, that large amounts of nitrogen are lost when nitrate is applied to apparently well aerated pasture soils.

The studies of Woldendorp are valuable because in them he examined the effects of microorganisms on denitrification at all levels of complexity, from pure cultures to sods sampled beneath perennial pasture.

E. Phosphate Release

That calcium phosphate is solubilized by isolates from the rhizosphere and other soil is well established (*99–101*), but there are conflicting reports on the relative proportions of phosphate-dissolving organisms in the rhizosphere and in the soil (*99–102*). The studies with isolates from the rhizosphere which have been grown in carbohydrate-rich media may have little bearing on the situation in the rhizosphere, where the carbohydrate supply would be limiting. Gerretson (*103*), however, showed that nonsterile plants dissolved and absorbed more phosphate from insoluble calcium phosphate than did sterile plants.

The use of a phosphate-dissolving strain of *Bacillus megatherium* as a

seed inoculum is widespread in the Soviet Union and is based upon the assumption that the organism proliferates in the rhizosphere and makes available soil phosphorus. Although Sundara Rao et al. (*104*) found that this organism increased the uptake of phosphate from both soil and fertilizer phosphate, Smith et al. (*105,106*) could find no difference in uptake between inoculated and uninoculated soils.

F. Polysaccharide Production

The existence of a mucilaginous sheath around plant roots has been discussed in Section 16-7. This material is produced aseptically by medic roots (*16*) and oat roots. Nevertheless, Webley et al. (*78*) found higher proportions of bacteria producing polysaccharide and slime in the rhizoplanes of several grasses than in soil away from roots. They suggested that grasses improve soil structure partly through polysaccharides produced by these rhizoplane inhabitants. An extension of such studies to assessments of the frequency of occurrence of slime-producing bacteria in the rhizospheres of plants which do not improve soil structure would be interesting, although their mere occurrence may not necessarily be the most important factor. The production of polysaccharide gums in culture is dependent upon an abundant supply of organic carbon in the medium, so the nature and quantities of root exudates would be important factors, too.

16-10. EFFECT OF RHIZOSPHERE MICROORGANISMS ON PLANTS

Most rhizosphere studies have concerned the effects of plants on soil microflora, and few have concerned the reverse effect. There is now increasing evidence that the rhizoplane and rhizosphere populations affect the host plants in many ways.

A. Root Development

During an investigation of the carbon dioxide evolution from sterile and nonsterile sunflower roots growing in nutrient solution Reuszer (*91*) found that the presence of microorganisms affected both the roots and the tops of the plants. The root weights were 7.07 and 2.51 g, and the top weights 21.83 and 11.27 g, of the sterile and nonsterile plants respectively. The nonsterile roots were yellow-brown and limp and tended to adhere together when removed from the flask, whereas the sterile roots were turgid and white.

Bowen and Rovira (*107*) found that the roots of tomato, subterranean clover, phalaris, and radiata pine were stunted by the presence of soil microorganisms in both agar and sand culture (Table 16-15). The degree of stunting was affected by environmental conditions and also by the nature

TABLE 16-15

Effects of Microorganisms on Root Growth in Sand (*107*)

Plant	Primary-root length, cm		Secondary-root length, cm	
	Sterile	Nonsterile	Sterile	Nonsterile
Subterranean clover	8.3	5.7[a]	8.5	5.6
Tomato	8.0	3.6[b]	15.4	10.0
Phalaris	24.4	14.6[a]	21.5	15.0[a]
Radiata pine	7.1	5.5[c]	7.1	4.3

[a] Significant at 1% level.
[b] Significant at 0.1% level.
[c] Significant at 5% level.

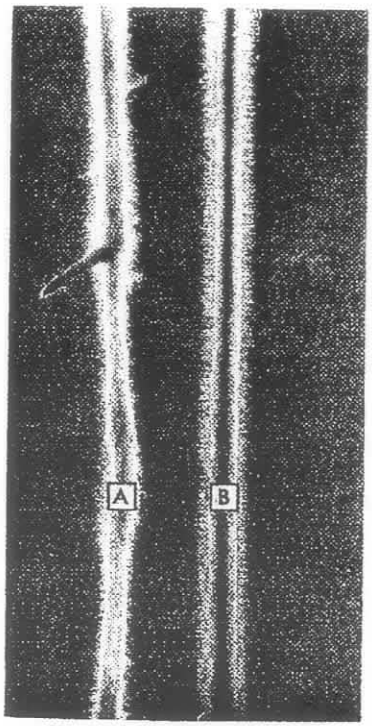

Fig. 16-10. Effects of microorganisms on growth of root hairs of subterranean clover: (A) nonsterile; (B) sterile. [From Bowen and Rovira (*107*).]

of the soil from which the inoculum was prepared. It was found that the inoculum of 0.1 ml of a 1-in-10 soil suspension consistently resulted in root-stunting, but higher dilutions seldom contained the active organisms, although in most of the soils tested the numbers of bacteria exceeded 10,000,000 per gram. Welte and Trolldenier (*108*) also observed the stunting of roots of red clover by soil inoculum in plant-nutrient solution, but the nonsterile plants gave significantly longer roots when the "solution" was made up with tricalcium phosphate and calcium sulfate of low solubility; these authors, too, concluded that inhibition was caused by components of the soil population.

Soil microorganisms can affect root-hair development (*107*); an example is shown in Fig. 16-10. Bowen and Rovira (*107*) showed that microorganisms retarded the initiation and growth of root hairs. Inocula from different soils had varying effects, and in most soils the causative organisms were present at dilutions of 1 in 100,000.

The mechanisms responsible for the effects of microorganisms on root and root-hair growth have not yet been elucidated. The fact that the effects do not occur when dilute suspensions are used as inocula (more than 1 in 100 for root-stunting and 1 in 100,000 for root-hair effects) indicates that they are caused by specific organisms and, if this is so, then they probably are due to organic substances produced by a minority of soil organisms. Proteins are known to influence root-hair development (*86*).

B. Composition of Root Sap

Krasil'nikov (*109*) found that sap from nonsterile cereals and legumes had a higher content of free amino acids than sap from sterile plants, but there were no differences in the patterns of amino acids. The general levels of amino acids were higher in the presence of organisms especially when either *Pseudomonas fluorescens* or a mixture of rhizosphere bacteria was the inoculum. This finding has been confirmed by Rempe and Kaltagova (*110*), whose results, summarized in Table 16-16, also show that the activities of some root enzymes and the chlorophyll contents of the tops were increased by rhizosphere microorganisms (cf. *128*).

In attempting to assess the contribution of the rhizosphere microflora itself to the enzymic activity of roots Rempe and Kaltagova estimated the enzymic activities of sterile roots with and without a suspension of rhizosphere bacteria, added immediately before enzyme analysis of the homogenate. The roots without added suspension, whose rhizosphere microflora had developed in situ during the growth of the plant, showed the greater enzymic activity. These results indicate that the rhizoplane microflora affects the enzymic activity of the root itself and that the greater

TABLE 16-16

Effects of Microorganisms on Enzyme Activity,[a] Amino Acid, and Chlorophyll Contents of Host Plants (*110*)

	Enzyme activity of roots						Amino acid, μg per g of dry matter		Chlorophyll, mg per 100 g of fresh leaves
	Invertase	Amylase	Protease	Catalase	Peroxidase	Polyphenol-oxidase	Roots	Tops	Tops
Sterile	200	51	0	1.7	1.0	0.3	1,490	3,399	223
Nonsterile	349	93	21	5.8	2.2	0.9	3,290	5,621	302

[a] Invertase activity in mg of reducing sugar per g fresh roots. Amylase activity in mg of maltose per g fresh roots. Protease activity in mm of diluted column of an 8% solution of gelatin. Peroxidase activity and polyphenol oxidase activity in ml of 0.01 N I_2 solution per g roots. Catalase activity in mg of H_2O_2 per g fresh roots.

activity of the nonsterile roots is not simply an additive effect due to the activity of the microorganisms which developed on the root.

C. Uptake of Nutrients

Given these effects of rhizosphere microorganisms upon root growth and sap composition it would be surprising if no differences were found in nutrient uptake between sterile and nonsterile roots.

1. Cation Uptake

Trolldenier and Marckwordt (*111*) measured the uptake from nutrient solution and the translocation of ^{86}Rb and ^{45}Ca in sterile and nonsterile red clover plants and found significantly higher contents per unit weight in the tops of the sterile plants. The total uptake, when expressed on a plant basis, was the same for sterile and nonsterile plants but, because the tops of the latter were heavier and their shoot-to-root weight ratios larger, the nonsterile plants were utilizing the elements more efficiently.

Similarly, Welte and Trolldenier (*112*) found that the ash contents, calculated on a dry weight basis, of the tops and roots of sterile red clover plants were higher than those of corresponding nonsterile plants. A recalculation of their results as total ash per gram of root has shown that the nonsterile plants took as much as 64% more nutrients from nutrient culture solutions than did the sterile ones, indicating a greater efficiency of the stunted nonsterile roots (*113*).

2. Anion Uptake

The results of studies of the effects of rhizosphere microorganisms on the anion uptake of roots are not as consistent as those of cation uptake.

Subba Rao et al. (*114*) reported that the tops of tomatoes with roots recently infected with *Fusarium* contained less ^{32}P, ^{35}S, and ^{14}C than did the tops of sterile plants when grown in solutions containing ^{32}P–phosphate, ^{35}S–sulfate, and ^{14}C–bicarbonate. These results refer to the amounts of nutrients translocated, since the total uptakes of the infected and uninfected plants were not measured. Similarly, Barber (*115*) found that, when 1-month-old barley roots were exposed for 24 hours to a 3×10^{-8} *M* phosphate solution containing ^{32}P–phosphate, sterile plants had double the uptake of nonsterile and also showed a much greater translocation. At a 3×10^{-4} *M* concentration there were no differences between them in either uptake or translocation. On the other hand, in short-term experiments with 5-day-old tomato and 8-day-old subterranean clover plants, Bowen and Rovira (*113*) found the total uptake greater in the nonsterile tomato by 85% and in the nonsterile clover by 45% compared with sterile plants; furthermore the percentage of phosphate translocated to the tops of nonsterile plants was greater, indicating a direct effect of micro-

organisms upon translocation. In this study the techniques for measuring the kinetics of uptake were followed; that is, the roots were washed in 5×10^{-4} *M* calcium sulfate before immersion in a solution of 5×10^{-4} *M* calcium sulfate and 5×10^{-6} *M* potassium dihydrogen phosphate containing ^{32}P-phosphate. The uptake periods ranged from 5 to 60 minutes.

Considering the differences in plant species, plant ages, pretreatment of roots, phosphate concentrations, and treatment times used in the various studies by different workers it is not surprising that the results should differ. The only conclusion which can be drawn is that microorganisms colonizing root surfaces can markedly influence phosphate uptake, and further experiments must be conducted over a range of phosphate levels under well-defined and reproducible conditions for short and long periods in soil, sand, and nutrient culture solutions.

D. Physiological Development and Crop Yields

The widespread use of *Azotobacter chroococcum* as a seed inoculum in the Soviet Union (*116,117*) has led to considerable study of its effects on the higher plants. Investigations with unsterilized soils have shown that the organism causes wheat to come into ear earlier (*25,118*) and causes the first truss of tomatoes to flower and produce ripe fruit earlier (*119*). A similar advancement in plant growth has been obtained with seeds inoculated with *Clostridium pasteurianum* and *Bacillus polymyxa* (*25,118*).

Yield increases are frequently obtained by inoculating seed with *Azotobacter* and other organisms (*25,117,120*) and, although marginal in terms of statistical significance, they occur often enough to indicate that microorganisms in the rhizosphere can influence crop yields.

The mechanisms by which the organisms affect plant growth are not yet fully elucidated. Jackson et al. (*119*) have correlative evidence that the response of tomatoes to *Azotobacter* inoculation is due to gibberellin-like substances; Krasil'nikov (*26*) has also suggested that substances promoting plant growth may be responsible, but considers that competition with inhibitory soil organisms and plant pathogens may be another factor.

Increased yields following seed inoculation have been obtained with a wide variety of organisms that originate in control soil and rhizosphere (*117,121*), indicating that in uninoculated soils the natural rhizosphere microflora may well be affecting plant development and yields. After studying pure cultures of twenty-five saprophytic fungi and five plant species Domsch (*122*) put forward the hypothesis that there are stimulatory and inhibitory fungi and that in natural soil the two groups balance each other; thus it appears that seed inoculation reduces the effects of inhibitory organisms by competing with them or else increases the number of stimulatory organisms in the rhizosphere.

16-11. MODIFICATION OF THE RHIZOSPHERE MICROFLORA

In the preceding section it was shown that rhizosphere microorganisms may be either detrimental or beneficial to higher plants, which raises the question of the possibility of modifying the population to improve plant growth.

A. Seed and Root Inoculation

Brown et al. (*123*) have shown that *Azotobacter* can be established in the rhizosphere of a variety of plants by inoculation of the seeds or roots. In field trials in South Australia similar conditions were found after the inoculation of wheat seed with *Azotobacter* and *Clostridium*, and although the numbers are not high compared with the general rhizosphere population, they are considerably greater than in the rhizosphere of uninoculated wheat (Rovira, unpublished results). *Azotobacter* in South Australian wheat soils are so low that they seldom appear on nitrogen-free agar medium, so that it is possible to study their distribution over the root systems of the plants simply by making impressions of the roots on the agar. In Figure 16-11 the *Azotobacter* appear as black mucoid colonies spreading from the site of inoculation (seed) to seminal roots and occasionally to nodal roots.

When it is considered that in spite of its being a poor colonizer of plant roots *Azotobacter* can spread throughout a root system, it is quite conceivable that inoculating seed with highly competitive organisms of the *Pseudomonas* or *Agrobacterium* type could alter the nature of the rhizosphere population.

B. Foliar Sprays

Demonstrations that foliar spraying with urea (*124,125*), antibiotics (*126*), and exogenous plant-growth regulators (*127*) modify root exudates and, sometimes, the balance of organisms in the rhizosphere indicate that this may well be used to inhibit root pathogens or to stimulate a rhizosphere microflora which is beneficial to plant growth. However, more must be known of the fundamental processes involved in foliar absorption, translocation, metabolism, effects on tops and roots and, finally, exudation into the rhizosphere, before foliar sprays will successfully control the rhizosphere microflora.

16-12. CONCLUSIONS

This review has described the microbiological and biochemical changes which occur in soil surrounding plant roots. That the rhizosphere is a zone of intense microbial activity is indisputable, but its significance in terms of the welfare of the host plant under field conditions has yet to be assessed.

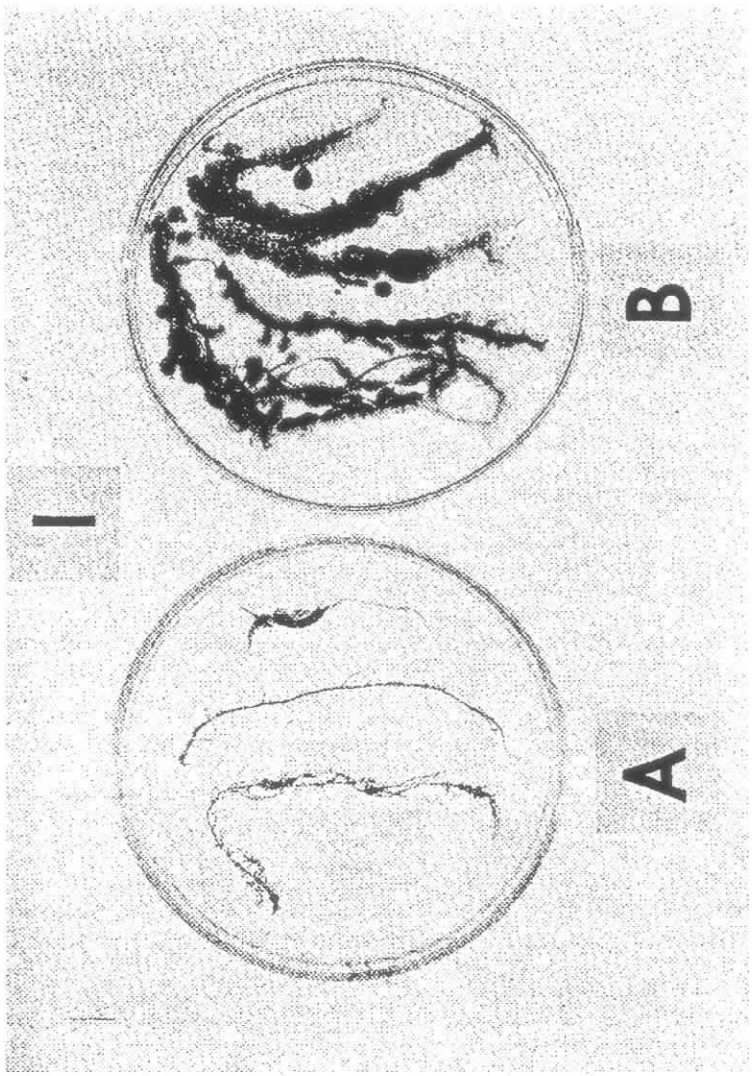

Fig. 16-11. Development of *Azotobacter* on seminal roots of barley grown in a sandy field soil for 6 weeks: (A) roots from uninoculated seed; (B) roots from *Azotobacter* inoculated seed.

Unfortunately, our knowledge of the effects of rhizoplane and rhizosphere microorganisms upon their host plant is both fragmentary and inconclusive. In any assessment of the significance of the rhizosphere in soil biochemistry it should be remembered that most of the soil under a grass sward and much of it under an advanced cereal crop must be considered to be rhizosphere.

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REFERENCES

1. H. Katznelson, in *Ecology of Soil-Borne Plant Pathogens—Prelude to Biological Control* (K. F. Baker and W. C. Snyder, eds.), Univ Calif. Press, Berkeley, 1965, p. 187.
2. A. D. Rovira, *Ann. Rev. Microbiol.*, **19**, 241 (1965).
3. H. Katznelson, *Soil Sci.*, **62**, 343 (1946).
4. F. E. Clark, *Soil Sci. Soc. Am. Proc.*, **12**, 239 (1947).
5. A. D. Rovira, *Australian Soil Sci. Conf., Adelaide*, 1953.
6. A. D. Rovira and W. R. Stern, *Australian J. Agr. Res.*, **12**, 1108 (1961).
7. S. Ishizawa, T. Susuki, O. Sato, and H. Toyoda, *Soil Plant Food (Tokyo)*, **3**, 85 (1957).
8. H. A. Louw and D. M. Webley, *J. Appl. Bacteriol.*, **22**, 216 (1959).
9. J. L. Harley and J. S. Waid, *Trans. Brit. Mycol. Soc.*, **38**, 104 (1955).
10. D. Parkinson, G. S. Taylor, and R. Pearson, *Plant Soil*, **19**, 332 (1963).
11. J. W. Rouatt and H. Katznelson, *J. Appl. Bacteriol.*, **24**, 164 (1961).
12. M. E. Brown, S. K. Burlingham, and R. M. Jackson, *Plant Soil*, **17**, 309 (1962).
13. P. C. T. Jones and J. E. Mollison, *J. Gen. Microbiol.*, **2**, 54 (1948).
14. A. D. Rovira, *J. Appl. Bacteriol.*, **19**, 72 (1956).
15. H. Jenny and K. Grossenbacher, *Soil Sci. Soc. Am. Proc.*, **27**, 273 (1963).
16. P. J. Dart and F. V. Mercer, *Arch. Mikrobiol.*, **47**, 344 (1964).
17. D. Jones and E. Griffiths, *Plant Soil*, **20**, 232 (1964).
18. L. E. Casida, *Appl. Microbiol.*, **13**, 327 (1965).
19. H. Katznelson, in *The Ecology of Soil Fungi* (D. Parkinson and J. S. Waid, eds.), Liverpool Univ. Press, Liverpool, 1960, p. 192.
20. J. L. Harley and J. S. Waid, *Plant Soil*, **7**, 96 (1955).
21. J. W. Rouatt, M. Lechevalier, and S. A. Waksman, *Antibiot. Chemotherapy*, **1**, 185 (1951).
22. E. Strzelczyk, *Can. J. Micribiol.*, **7**, 507 (1961).
23. L. Varga, *Agrokem. Talajtan.*, **7**, 393 (1958).
24. V. E. Henderson and H. Katznelson, *Can. J. Microbiol.*, **7**, 163 (1961).
25. A. D. Rovira, *Plant Soil*, **19**, 304 (1963).
26. N. A. Krasil'nikov, *Soil Micro-Organisms and Higher Plants*, Academy of Sciences, USSR, Moscow, 1958, English transl.
27. E. C. S. Chan and H. Katznelson, *Can. J. Microbiol*, **7**, 759 (1961).
28. H. Katznelson, J. W. Rouatt, and T. M. B. Payne, *Proc. Intern. Soil Sci. Soc. Congr. 6th, Paris*, 1956, p. 151.
29. A. G. Lochhead and F. E. Chase, *Soil Sci.*, **55**, 185 (1943).
30. R. H. Wallace and H. DeL. King, *Soil Sci. Soc. Am. Proc.*, **18**, 282 (1954).
31. A. G. Lochhead and J. W. Rouatt, *Soil Sci. Soc. Am. Proc.*, **19**, 48 (1955).
32. A. C. Zagallo and H. Katznelson, *J. Bacteriol.*, **73**, 760 (1957).
33. A. D. Rovira, *Plant Soil*, **7**, 195 (1956).
34. A. D. Rovira and P. G. Brisbane, *Ecology of Soil Bacteria*, Univ. Liverpool Press, Liverpool, 1966.
35. R. M. Jackson, *Nature*, **180**, 96 (1957).

36. B. Catska and J. Macura, *Ust. Ved. Inf. M.Z.L.V.H., Rost. Vyroba*, **36**, 692 (1963).
37. M. N. Schroth and D. C. Hildebrand, *Ann. Rev. Phytopathy*, **2**, 101 (1964).
38. E. W. Buxton, *Trans. Brit. Mycol. Soc.*, **40**, 145 (1957).
39. D. Parkinson, in *Plant Microbes Relationships* (J. Macura and V. Vancura, eds.), Czech. Acad. Sci., Prague, 1965, p. 69.
40. F. E. Clark, *Trans. Kansas Acad. Sci.*, **43**, 75 (1940).
41. A. D. Rovira, *Australian J. Agr. Res.*, **12**, 77 (1961).
42. G. C. Papavizas and C. B. Davey, *Plant Soil*, **14**, 215 (1961).
43. H. R. Wallace, *Nematologica*, **3**, 236 (1958).
44. K. P. Barley, paper presented at the *Australian Mathematical Society Meeting, Adelaide*, May 1964.
45. K. P. Barley and R. H. Sedgley, *Australian J. Sci.*, **23**, 297 (1961).
46. J. Rivière, *Contribution à l'étude de la rhizosphere du blé*, D.Sc. thesis, Univ. Paris, 1959.
47. R. L. Starkey, *Soil Sci.*, **27**, 319 (1929).
48. A. D. Rovira, in *Ecology of Soil-Borne Plant Pathogens—Prelude to Biological Control* (K. F. Baker and W. C. Snyder, eds.), Univ. Calif. Press, Berkeley, 1965, p. 170.
49. G. W. Butler, R. M. Greenwood, and K. Soper, *New Zealand J. Agr. Res.*, **2**, 415 (1959).
50. A. D. Rovira, *Plant Soil*, **7**, 178 (1956).
51. A. D. Rovira, *Soils Fertilizers*, **25**, 167 (1962).
52. V. Vancura and A. Hovadik, in *Plant Microbes Relationships* (J. Macura and V. Vancura, eds.), *Czech. Acad. Sci., Prague*, 1965, p. 21.
53. M. V. Meshkov, *Works of Symposium on Soil Microbiology*, 1953, p. 285; through Krasil'nikov, *Soil Micro-Organisms and Higher Plants* (Ref. *26*).
54. G. W. Harmsen and G. Jager, *Nature*, **195**, 1119 (1962).
55. A. G. Norman, *Arch. Biochem. Biophys.*, **58**, 461 (1955).
56. P. Martin, *Arch. Mikrobiol.*, **29**, 154 (1958).
57. A. D. Rovira, *Plant Soil*, **11**, 53 (1959).
58. H. Katznelson, J. W. Rouatt, and T. M. B. Payne, *Plant Soil*, 7, 35 (1955).
59. M. N. Schroth and W. C. Snyder, *Phytopathology*, **51**, 389 (1961).
60. R. Pearson and D. Parkinson, *Plant Soil*, **13**, 391 (1961).
61. G. A. Zentmyer, *Science*, **133**, 1595 (1961).
62. A. F. Bird, *Nematologica*, **4**, 322 (1959).
63. A. F. Bird, *Nematologica*, **5**, 217 (1960).
64. B. Frenzel, *Planta*, **55**, 169 (1960).
65. A. I. Virtanen, S. von Hausen, and T. Laine, *J. Agr. Sci.*, **27**, 332 (1937).
66. O. Wyss and P. W. Wilson, *Soil Sci.*, **52**, 15 (1941).
67. G. W. Butler and N. O. Bathurst, *Proc. 7th Intern. Grassland Conf. Palmerston North, New Zealand*, 1956, p. 168.
68. G. D. Bowen, *Queensland J. Agr. Sci.*, **16**, 253 (1959).
69. N. S. Subba Rao, R. G. S. Bidwell, and D. L. Bailey, *Can. J. Botany*, **40**, 203 (1962).
70. V. P. Ivanov, G. A. Yakobsen, and B. S. Fomenko, *Fiziol. Rast.*, **11**, 630 (1964).
71. V. Slankis, V. C. Runeckles, and G. Krotkov, *Physiol. Plantarum.*, **17**, 301 (1964).
72. B. M. McDougall and A. D. Rovira, *Nature*, **207**, 1104, (1965).
73. E. A. Roberts, *Botan. Gaz.*, **62**, 488 (1916).
74. P. J. Linder, J. C. Craig, and T. R. Walton, *Plant Physiol.*, **32**, 572 (1957).
75. J. W. Mitchell, in *Conn. Agri. Expt. Sta. Bull. 663*, 1963, p. 48.
76. F. M. Scott, K. C. Hamner, E. Baker, and E. Bowler, *Am. J. Botany*, **45**, 449 (1958).

77. C. J. Dawes and E. Bowler, *Am. J. Botany*, **46**, 561 (1959).
78. D. M. Webley, R. B. Duff, J. S. D. Bacon, and V. C. Farmer, *J. Soil Sci.*, **16**, 149 (1965).
79. A. Frey-Wyssling and K. Mühlethaler, *Mikroskopie*, **4**, 257 (1949).
80. J. de Lopez-Gonzalez and H. Jenny, *J. Colloid. Sci.*, **14**, 533 (1959).
81. K. Esau, *Plant Anatomy*, Wiley, New York, 1962, pp. 117, 118, plate 72.
82. H. T. Rogers, R. W. Pearson, and W. H. Pierre, *Soil Sci. Soc. Am. Proc.*, **5**, 285 (1940).
83. H. T. Rogers, R. W. Pearson, and W. H. Pierre, *Soil Sci.*, **54**, 353 (1942).
84. C. W. Chang and R. S. Bandurski, *Plant Physiol.*, **39**, 60 (1964).
85. J. A. Hellebust and D. F. Forward, *Can. J. Botany*, **40**, 113 (1962).
86. J. M. Ulrich, R. A. Luse, and A. D. McLaren, *Physiol. Plantarum*, 17, 683 (1964).
87. G. Fåhraeus and H. Ljunggren, *Physiol. Plantarum*, **12**, 145 (1959).
88. H. Ljunggren and G. Fåhraeus, *J. Gen. Microbiol.*, **26**, 521 (1961).
89. M. R. Pollock, in *The Bacteria*, Vol. IV (I. C. Gunsalus and R. Y. Stanier, eds.), Academic Press, New York, 1962, p. 121.
90. R. Davies, in *Biochemistry of Industrial Microorganisms* (C. Rainbow and A. H. Rose, eds.), Academic Press, New York, 1963, p. 68.
91. H. W. Reuszer, *Soil Sci. Soc. Am. Proc.*, **14**, 175 (1949).
92. H. Katznelson and J. W. Rouatt, *Can. J. Microbiol.*, **3**, 673 (1957).
93. A. D. Rovira, *Factors Influencing Soil Metabolism and the Relationship between Plant Roots and Soil Micro-Organisms*, Ph.D. thesis, Univ. Sydney, 1955.
94. A. D. Rovira, *Plant Soil*, **7**, 209, (1956).
95. R. L. Starkey, *Soil Sci.*, **27**, 433 (1929).
96. C. A. I. Göring and F. E. Clark, *Soil Sci. Soc. Am. Proc.*, **13**, 261 (1948).
97. J. A. E. Molina and A. D. Rovira, *Can. J. Microbiol.*, **10**, 249 (1964).
98. J. W. Woldendorp, *Mededel. Landbouwhogeschool Wageningen*, **63**(13), 1, (1963).
99. J. I. Sperber, *Australian J. Agr. Res.*, **9**, 782 (1958).
100. H. Katznelson and B. Bose, *Can. J. Microbiol.*, **5**, 79 (1959).
101. H. A. Louw and D. M. Webley, *J. Appl. Bacteriol.*, **22**, 227 (1959).
102. H. A. Louw and A. Erasmus, *S. African J. Lab. Clin. Med.*, **10**, 32 (1964).
103. F. C. Gerretsen, *Plant Soil*, **1**, 51 (1948).
104. W. V. B. Sundara Rao, P. D. Bajpai, J. P. Sharm, and B. V. Subbiah, *J. Indian Soc. Soil Sci.*, **11**, 209 (1963).
105. J. H. Smith, F. E. Allison, and D. A. Soulides, *Soil Sci. Soc. Am. Proc.*, **25**, 109 (1961).
106. J. H. Smith, F. E. Allison, and D. A. Soulides, *U.S. Dept. Agr. Tech. Bull. 1263*, 1962.
107. G. D. Bowen and A. D. Rovira, *Plant Soil*, **15**, 166 (1961).
108. E. Welte and G. Trolldenier, in *Plant Microbes Relationships* (J. Macura and V. Vancura, eds.), Czech. Acad. Sci., Prague, 1965, p. 186.
109. N. Krasil'nikov, *J. Gen. Appl. Microbiol. (Tokyo)*, **7**, 128 (1961).
110. J. K. Rempe and O. G. Kaltagova, in *Plant Microbes Relationships* (J. Macura and V. Vancura, eds.), Czech. Acad. Sci., Prague, 1965, p. 178.
111. G. Trolldenier and U. Marckwordt, *Arch. Mikrobiol.*, **43**, 148 (1962).
112. E. Welte and G. Trolldenier, *Arch. Mikrobiol.*, **43**, 138 (1962).
113. G. D. Bowen and A. D. Rovira, *Nature*, **211**, 665 (1966).
114. N. S. Subba Rao, R. G. S. Bidwell, and D. L. Bailey, *Can. J. Botany*, **39**, 1759 (1961).
115. D. A. Barber, in *Ann. Rept. Agricultural Research Council, Radiobiological Laboratory, Wantage, England*, 1965.

116. R. Cooper, *Soils Fertilizers*, **22**, 327 (1959).
117. E. N. Mishustin and A. N. Naumova, *Mikrobiologiya*, **31**, 543 (1962); p. 442 of English transl.
118. A. D. Rovira, in *Plant Microbes Relationships* (J. Macura and V. Vancura, eds.), Czech. Acad. Sci., Prague, 1965, p. 193.
119. R. M. Jackson, M. E. Brown, and S. K. Burlingham, *Nature*, **203**, 851 (1964).
120. M. E. Brown, S. K. Burlingham, and R. M. Jackson, *Plant Soil*, **20**, 194 (1964).
121. G. Pantos, *Agrokem. Talajtan.*, **5**, 351 (1956).
122. K. H. Domsch, *Z. Pflanzenkrankh. Pflanzenschutz*, **70**, 470 (1963).
123. M. E. Brown, S. K. Burlingham, and R. M. Jackson, *Plant Soil*, **17**, 320 (1962).
124. V. P. Agnihotri, *Plant Soil*, **20**, 364 (1964).
125. R. K. Horst and L. J. Herr, *Phytopathology*, **52**, 423 (1962).
126. J. Vrany, V. Vancura, and J. Macura, *Folia Microbiol. (Prague)*, 7, 61 (1962).
127. P. J. Linder, J. W. Mitchell, and G. D. Freeman, *J. Agr. Food Chem.*, **12**, 437 (1964).
128. E. F. Estermann and A. D. McLaren, *Plant Soil*, **15**, 243 (1961).